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Paper No.:____

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Inventor(s): HUANG, Dong; QI, Dong Feng
Title: NOVEL AGLYCON DAMMARANE SAPOGENINS, THEIR USE AS ANTI-CANCER AGENTS, AND A PROCESS FOR PRODUCING SAME
Serial No.: 09/910887
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Examiner: Qazi, Sabiha Naim Art Unit: 1616
Date: 10 May 2004

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

AFFIDAVIT UNDER RULE 1.132

I, Dong Huang, of 16788 102 Avenue, Surrey, British Columbia, Canada, V4N 4X2, MAKE OATH AND SAY AS FOLLOWS:

1. I have personal knowledge of the matters sworn to herein, except where the matters are stated to be based on information and belief, in which case I believe them to be true.

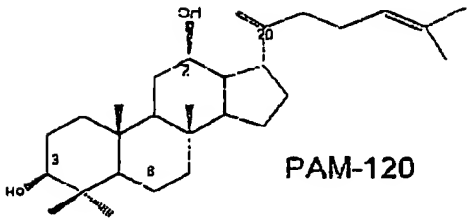
2. I am a co-inventor of the invention described and claimed in US Patent Application Serial No. 09/910887.

3. I hold a Bachelor of Science degree from the University of Beijing in China.

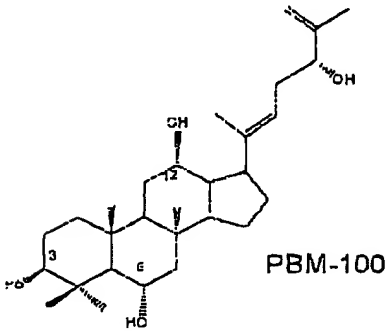
4. I have over 20 years of experience in the fields of botany chemistry research and ginsenoside drug development.

5. I have conducted side-by-side experiments to compare the efficacy of the compounds PAM-120, PBM-100, Rg3, and Rh2.

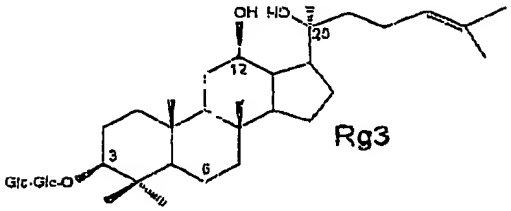
6. PAM-120 has the following formula:



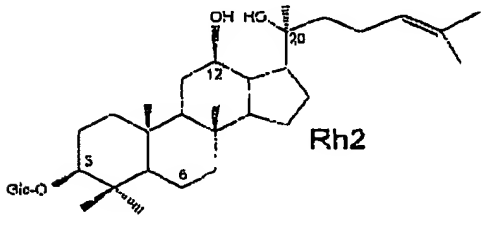
PBM-100 has the following formula:



Rg3 has the following formula:



Rh2 has the following formula:



7. I conducted experiments to compare the efficacy of PAM-120, PBM-100, Rg3, and Rh2 against lung cancer cells in the following manner. Compounds PAM-120, PBM-100, Rg3, and Rh2 were obtained from Pegasus Pharmaceuticals Group Inc. Human non-small-cell H460 lung cancer cells were seeded at $3x10^4$ cells/well in a 96-well plate in DMEM medium supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO_2. The cells were treated with isolated ginsenoside Rg3, Rh2, PAM-120, and PBM-100 at a fixed dose of 25 uM. The cytotoxic effects of the compounds on the lung cancer cells were measured by determining the viability of the cells. Cell viability was measured using the MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) assay method (Denizot and Kang, J. Immunol. Meth. 89:271-277 (1986); Carmichael et al., Cancer Res. 47:936-942 (1987)) 24 hours following treatment. Cell viability is measured by determining the absorbency of stained cells. Non-viable cells have lower absorbency compared to viable cells. Table I shows the viability of H460 lung cancer cells in the presence of the compounds Rg3, Rh2, PAM-120, and PBM-100 at 25 uM.

Compound (25 uM)	Absorbency of stained cells (+/- SD)	Viability (%)
Blank control	0.368 +/- 0.069	100.00
Rg3	0.298 +/- 0.071	80.98
Rh2	0.278 +/- 0.030	78.49
PAM-120	0.220 +/- 0.051	62.08
PBM-100	0.223 +/- 0.040	62.72

Table 1: Viability of H460 Lung Cancer Cells in the Presence of 25uM Rg3, Rh2, PAM-120 and PBM-100.

8. The results in Table 1 illustrate that H460 lung cancer cells are significantly less viable in the presence of PAM-120 and PBM-100 than either Rg3 or Rg2. Therefore, PAM-120 and PBM-100 have greater cytotoxic effects than either Rg3 or Rh2.

9. I conducted experiments to compare the efficacy of PAM-120, PBM-100, Rg3, and Rh2 against breast cancer cells in the following manner. Compounds PAM-120, PBM-100, Rg3, and Rh2 were obtained from Pegasus Pharmaceuticals Group Inc. Human drug resistant MCF7r breast cancer cells were seeded at $3x10^4$ cells/well in a 96-well plate in DMEM medium supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO_2. The cells were treated with isolated ginsenoside Rg3, Rh2, PAM-120 and PBM-100 at various concentrations. The IC50s of the compounds Rg3, Rh2, PAM-120, and PBM-100 were determined using standard methods. IC50 is the concentration of a compound needed to reduce the growth of a population of cells by 50%. The IC50s of the compounds are shown in Table 2.

Compound	IC_{50} (ug/ml)
Rg3	67.5 +/- 9.0
Rh2	35.2 +/- 4.3
PAM-120	<10
PBM-100	15 3 +/- 2.3

Table 2: IC50 Values of Compounds PAM-120, PBM-100, Rg3, and Rh2 Against MCF7r Breast Cancer Cells.

10. The results in Table 2 illustrate that PAM-120 and PBM-100 have significantly lower IC50 concentrations than Rg3 and Rh2. PAM-120 has an IC50 nearly 7 times less than the IC50 of Rg3 and nearly 4 times less than the IC50 of Rh2. PBM-100 has an IC50 nearly 4.5 times less than the IC50 of Rg3 and nearly 2.5 times less than the IC50 of Rh2. Therefore, PAM-120 and PBM-100 are effective at inhibiting MCF7r breast cancer cells at significantly lower concentrations than either Rg3 or Rh2.

11. I conducted experiments to compare the efficacy of PAM-120, PBM-100, Rg3, and Rh2 against melanoma cells in the following manner. Compounds PAM-120, PBM-100, Rg3, and Rh2 were obtained from Pegasus Pharmaceuticals Group Inc. Mouse B16 melanoma cells were seeded at $3x10^4$ cells/well in a 96-well plate in DMEM medium supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO_2. The cells were treated with isolated ginsenoside Rg3, Rh2, PAM-120 and PBM-100 at various concentrations. The IC50s of the compounds Rg3, Rh2, PAM-120, and PBM-100 were determined using standard methods. The IC50s of the compounds are shown in Table 3.

Compound	IC50 (ug/mL)
Rg3	30.2 +/- 4.6
Rh2	28.1 +/- 4.9
PAM-120	<10
PBM-100	<10

Table 3: IC50 Values of Compounds PAM-120, PBM-100, Rg3, and Rh2 Against B16 Melanoma Cells.

12. The results in Table 3 illustrate that both PAM-120 and PBM-100 have IC50 values nearly 3 times lower than the IC50 values of Rg3 and Rh2. Therefore, PAM-120 and PBM-100 are effective at inhibiting melanoma cells at significantly lower concentrations than Rg3 and Rh2.

SWORN before me at the)
city of Surrey, in the)
Province of British Columbia,)
Canada this 10 day of May, 2004)
)
)
A Notary Public in and for the)
Province of British Columbia,)
Canada. My Commission is for life.)

Dong Huang

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